

SUPPORTING INFORMATION

Quaternary Ammonium Compounds: Bioaccumulation Potentials in Humans and Levels in Blood before and during the COVID-19 Pandemic

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Chemicals and reagents. All native standards were purchased from Sigma-Aldrich, including benzylidimethyloctylammonium chloride (C8-BAC), benzylidimethyldecylammonium chloride (C10-BAC), benzylidimethyldodecylammonium chloride (C12-BAC), benzylidimethyltetradecylammonium chloride (C14-BAC), benzylidimethylhexadecylammonium chloride (C16-BAC), stearyldimethylbenzylammonium chloride (C18-BAC), dioctyldimethylammonium bromide (C8-DDAC), didecyldimethylammonium bromide (C10-DDAC), didodecyldimethylammonium bromide (C12-DDAC), dimethylditetradecylammonium bromide (C14-DDAC), dihexadecyldimethylammonium bromide (C16-DDAC), dimethyldioctadecylammonium bromide (C18-DDAC), octyltrimethylammonium chloride (C8-ATMAC), decyltrimethylammonium bromide (C10-ATMAC), dodecyltrimethylammonium chloride (C12-ATMAC), tetradecyltrimethylammonium chloride (C14-ATMAC), hexadecyltrimethylammonium chloride (C16-ATMAC), and octadecyltrimethylammonium chloride (C18-ATMAC). Two labeled standards, benzylidimethyldodecylammonium-d₇ chloride (d₇-C12-BAC) and benzylidimethyltetradecylammonium-d₇ chloride (d₇-C14-BAC), were obtained from Toronto Research Chemicals. Another labeled standard, n-decyltrimethylammonium-d₉ chloride (d₉-C10-ATMAC), was purchased from Santa Cruz Biotechnology. Stock solutions of each QAC were prepared in acetonitrile. All solvents used in this study were Optima grade. Mixed gender human liver microsomes (pool size = 50) were obtained from Sekisui XenoTech, Oasis WCX cartridges (6cc, 150mg, 30μm) were from Waters and the NADPH regeneration system was from Promega.

Table S1. The optimized MRM transitions, fragmentors, and collision energies for target analytes.

Compound	Abbreviation	Retention time (min)	Precursor ion [M-Cl/Br] ⁺	Fragmentor (volts)	Product ions (m/z)	Collision energy (volts)
Benzyltrimethylammonium chloride	C8-BAC	3.53	248.2	103	91 65.1	29 77
Benzyltrimethyldecylammonium chloride	C10-BAC	4.12	276.3	103	91.1 184	33 21
Benzyltrimethyldodecylammonium chloride	C12-BAC	4.65	304.3	113	91 212	41 25
Benzyltrimethyltetradecylammonium chloride	C14-BAC	5.13	332.3	122	91.1 240	41 25
Benzyltrimethylhexadecylammonium chloride	C16-BAC	5.53	360.4	146	91.1 268	41 25
Stearyltrimethylbenzylammonium chloride	C18-BAC	5.85	388.39	127	296.3 91	29 45
Dioctyltrimethylammonium bromide	C8-DDAC	4.46	270.3	156	158.2 71.1	29 33
Didecyldimethylammonium bromide	C10-DDAC	5.31	326.4	151	186 71.1	33 37
Didodecyldimethylammonium bromide	C12-DDAC	5.93	382.4	181	214 71.1	37 41
Dimethylditetradecylammonium bromide	C14-DDAC	6.35	438.5	151	242 71.1	41 49
Dihexadecyldimethylammonium bromide	C16-DDAC	6.63	494.6	151	270 71.1	49 53
Dimethyldioctadecylammonium bromide	C18-DDAC	7.08	550.6	175	298 71.1	53 57
Octyltrimethylammonium chloride	C8-ATMAC	2.73	172.2	132	85.1 71.1	21 25
Decyltrimethylammonium bromide	C10-ATMAC	3.48	200.2	127	85.1 71.1	21 25
Dodecyltrimethylammonium chloride	C12-ATMAC	4.09	228.3	137	85.1 71.1	25 25
Tetradecyltrimethylammonium chloride	C14-ATMAC	4.67	256.3	142	85.1 71.1	29 29
Hexadecyltrimethylammonium chloride	C16-ATMAC	5.18	284.3	132	85.1 71.1	29 33
Octadecyltrimethylammonium chloride	C18-ATMAC	5.61	312.4	142	85.1 71.1	33 33
Benzyltrimethyldodecylammonium-d7 chloride (Surrogate standard)	d ₇ -C12-BAC	4.63	311.34	122	98.1 212	37 25
n-decyltrimethylammonium-d ₉ chloride (Surrogate standard)	d ₉ -C10-ATMAC	3.48	209.3	127	85.1 71.1	21 25
(Benzyl-d7)dimethyltetradecylammonium chloride (Internal standard)	d ₇ -C14-BAC	5.11	339.38	127	98.1 70.1	41 97

Table S2. Procedural and field blank levels and method detection limits (MDL, ng/mL). ND: non detects.

QACs	Procedural blanks	Field blanks	MDL
C8-BAC	ND	ND	0.01
C10-BAC	ND	ND	0.01
C12-BAC	0.08	0.04	0.02
C14-BAC	0.09	0.10	0.10
C16-BAC	ND	ND	0.01
C18-BAC	ND	ND	0.01
C8-DDAC	ND	ND	0.01
C10-DDAC	0.09	0.06	0.13
C12-DDAC	ND	ND	0.01
C14-DDAC	0.35	0.43	0.37
C16-DDAC	ND	ND	0.01
C18-DDAC	ND	ND	0.01
C8-ATMAC	ND	ND	0.01
C10-ATMAC	ND	ND	0.01
C12-ATMAC	0.25	0.27	0.10
C14-ATMAC	0.35	0.15	0.15
C16-ATMAC	0.12	0.11	0.17
C18-ATMAC	ND	ND	0.01

Table S3. Average matrix spike recoveries for each target analyte (%). SE: standard error.

QAC	Mean	SE
C8-BAC	62	0.9
C10-BAC	74	1.1
C12-BAC	118	3.6
C14-BAC	127	0.4
C16-BAC	89	1.3
C18-BAC	95	2.1
C8-DDAC	81	1.0
C10-DDAC	82	1.3
C12-DDAC	98	2.0
C14-DDAC	112	1.8
C16-DDAC	126	1.8
C18-DDAC	87	1.2
C8-ATMAC	84	1.8
C10-ATMAC	80	1.9
C12-ATMAC	86	1.7
C14-ATMAC	103	1.1
C16-ATMAC	95	1.3
C18-ATMAC	98	1.9

Table S4. Average surrogate standard recoveries in analyzed samples (%). SE: standard error.

Compounds	Mean	SE
d ₇ -C12-BAC	108	4.8
d ₉ -C10-ATMAC	117	5.0

Table S5. The nonspecific binding (NSB, unitless) to the ultra-centrifugal filter at different substrate concentrations (0.25, 0.5 and 1 μM).

	0.25 μM	0.5 μM	1 μM
C8-BAC	0.54 \pm 0.06	0.72 \pm 0.08	0.6 \pm 0.07
C10-BAC	0.59 \pm 0.07	0.75 \pm 0.08	0.66 \pm 0.07
C12-BAC	0.79 \pm 0.09	0.84 \pm 0.09	0.78 \pm 0.09
C14-BAC	0.89 \pm 0.12	0.88 \pm 0.10	0.88 \pm 0.13
C16-BAC	0.89 \pm 0.11	0.88 \pm 0.17	0.89 \pm 0.12
C18-BAC	0.89 \pm 0.17	0.88 \pm 0.11	0.89 \pm 0.11
C8-DDAC	0.79 \pm 0.09	0.85 \pm 0.09	0.82 \pm 0.09
C10-DIAA	0.89 \pm 0.15	0.89 \pm 0.10	0.89 \pm 0.15
C12-DIAA	0.89 \pm 0.18	0.88 \pm 0.13	0.89 \pm 0.12
C14-DIAA	0.89 \pm 0.13	0.88 \pm 0.11	0.89 \pm 0.10
C16-DIAA	0.90 \pm 0.14	0.89 \pm 0.11	0.90 \pm 0.14
C18-DIAA	0.90 \pm 0.18	0.89 \pm 0.12	0.90 \pm 0.16
C8-ATMAC	0.67 \pm 0.07	0.74 \pm 0.08	0.72 \pm 0.08
C10-ATMAC	0.65 \pm 0.07	0.78 \pm 0.09	0.71 \pm 0.08
C12-ATMAC	0.64 \pm 0.07	0.78 \pm 0.09	0.72 \pm 0.08
C14-ATMAC	0.78 \pm 0.09	0.84 \pm 0.09	0.82 \pm 0.09
C16-ATMAC	0.89 \pm 0.14	0.89 \pm 0.11	0.89 \pm 0.10
C18-ATMAC	0.89 \pm 0.15	0.89 \pm 0.12	0.89 \pm 0.10

Table S6. Calculated binding affinities to serum proteins (SPB, %) and blood to serum ratios (B/S, unitless) for each targeted QAC.

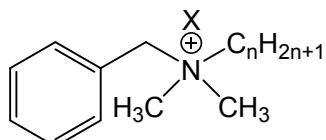
QACs	SPB	B/S
<i>BACs</i>		
C8-BAC	50 \pm 6.2	2.18
C10-BAC	92 \pm 1.2	1.68
C12-BAC	93 \pm 0.4	1.48
C14-BAC	70 \pm 2.7	1.98
C16-BAC	65 \pm 7.0	2.01
C18-BAC	60 \pm 12	2.86
<i>DDACs</i>		
C8-DDAC	63 \pm 1.2	3.77
C10-DDAC	73 \pm 2.0	3.39
C12-DDAC	72 \pm 9.3	3.15
C14-DDAC	43 \pm 11	4.64
C16-DDAC	50 \pm 12	7.59
C18-DDAC	34 \pm 11	16.5
<i>ATMACs</i>		
C8-ATMAC	14 \pm 6.5	3.02

C10-ATMAC	67 ± 3.9	2.72
C12-ATMAC	94 ± 0.7	2.29
C14-ATMAC	55 ± 5.5	2.64
C16-ATMAC	56 ± 5.2	3.88
C18-ATMAC	69 ± 6.9	4.39

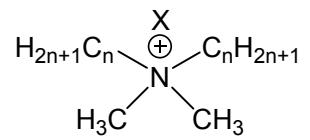
Table S7. Summary of demographic characteristics for the study participants ($n = 222$). BMI: body mass index.

Parameters	N	Percentage, %	N	Percentage, %
			Before COVID-19 (n=111)	
Age (years)	53	48	53	48
Gender	58	52	58	52
	55	50	55	50
Race	56	50	56	50
	White/Caucasian	95	103	93
Smoking	African American	14	8	7
	Other	2	0	0
Smoking	Smoker	41	73	66
	Non-smoker	55	37	33
BMI (kg/m ²)	Missing	15	1	1
	Underweight, <18.5	2	2	2
	Normal, 18.5–24.9	21	17	15
	Overweight, 25–29.9	46	33	30
	Obese, >30	42	59	53

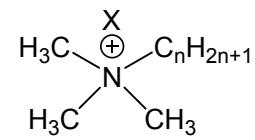
X: chloride or bromide



n=6, 8, 10, 12, 14, 16, 18



n=8, 10, 12, 14, 16, 18



n=8, 10, 12, 14, 16, 18

Figure S1. Chemical structures of the three QAC groups targeted in this study.

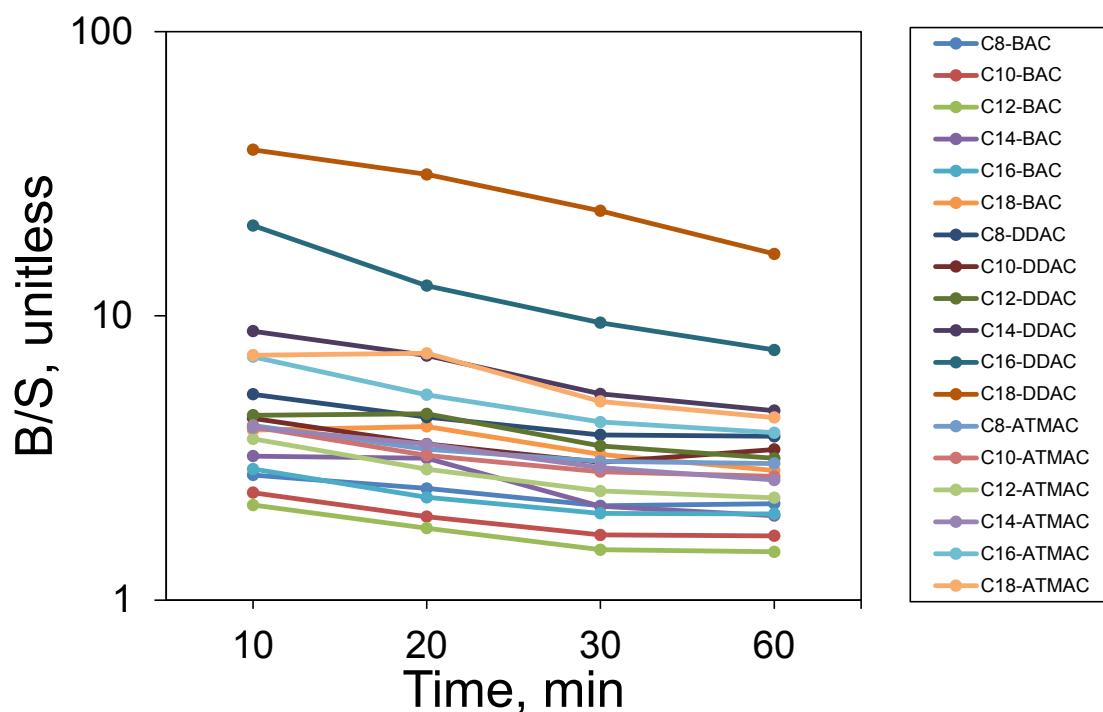


Figure S2. Blood to serum ratios (B/S, unitless) at various incubation times (min) .

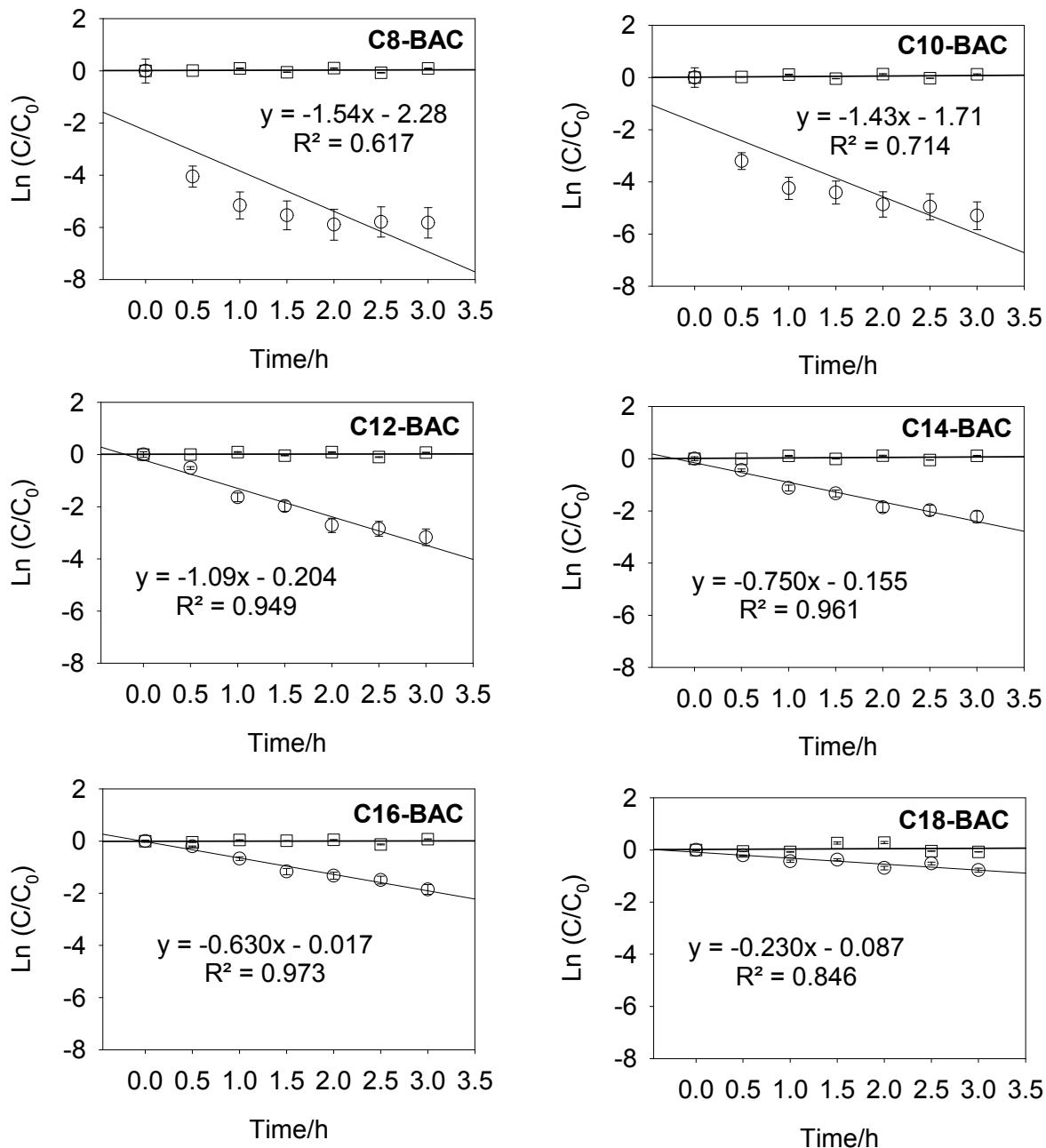


Figure S3. Substrate depletion curves for BACs (squares: control samples; circles: experimental samples). The error bars indicate the standard deviation of the triplicate tests. When not visible, error bars are contained within the data point.

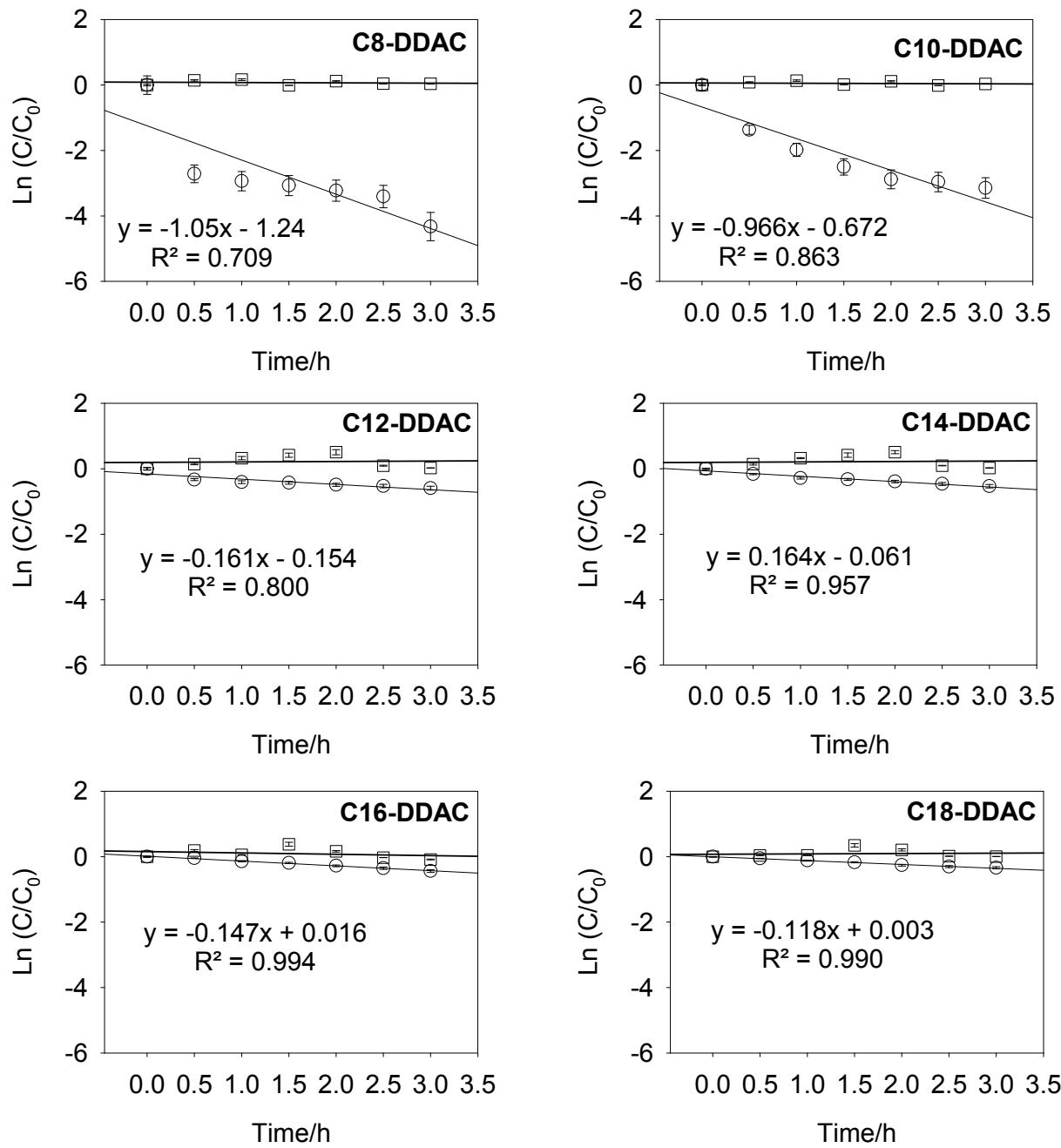


Figure S4. Substrate depletion curves for DDACs (squares: control samples; circles: experimental samples). The error bars indicate the standard deviation of the triplicate tests. When not visible, error bars are contained within the data point.

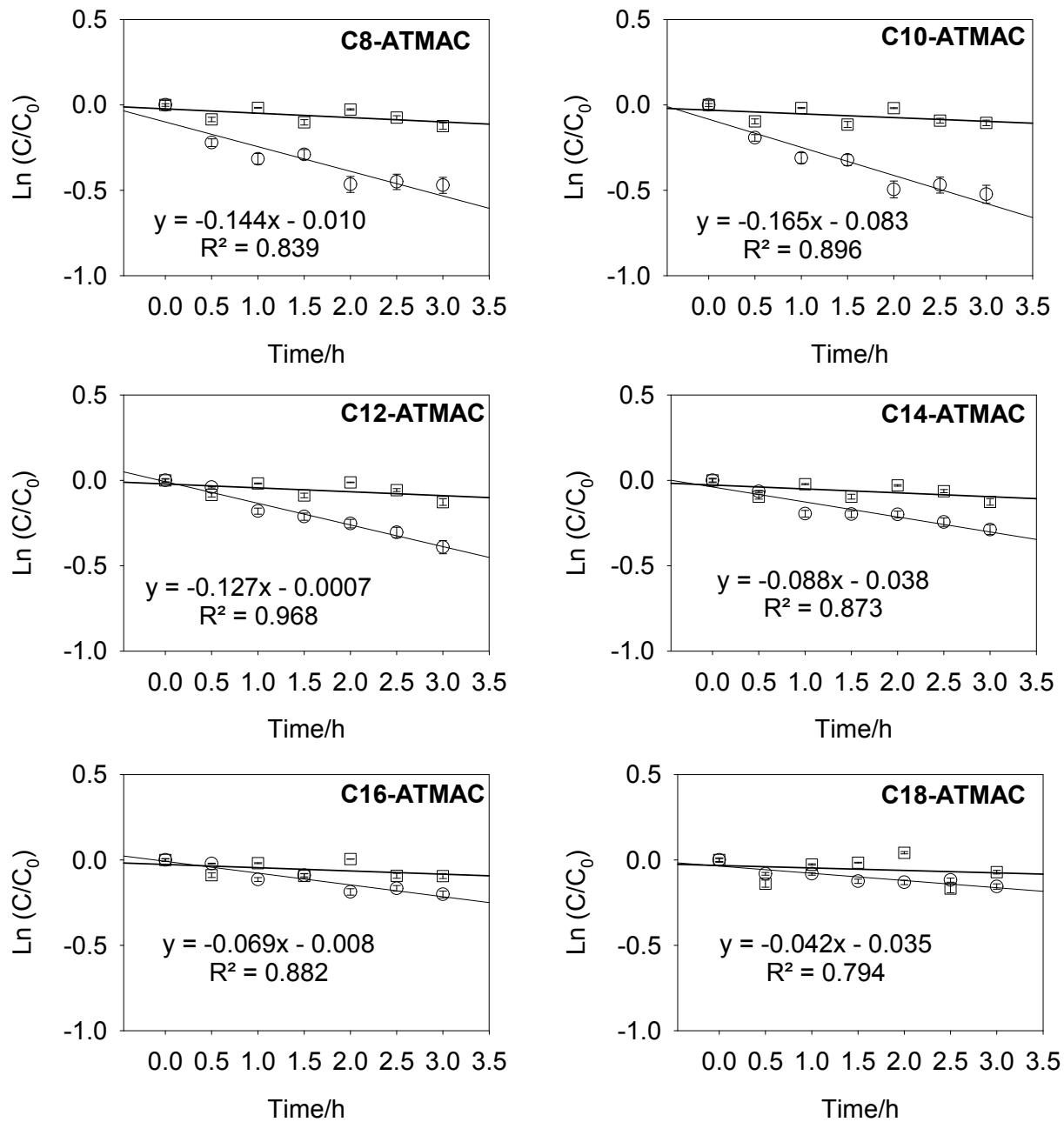


Figure S5. Substrate depletion curves for ATMACs (squares: control samples; circles: experimental samples). The error bars indicate the standard deviation of the triplicate tests. When not visible, error bars are contained within the data point.

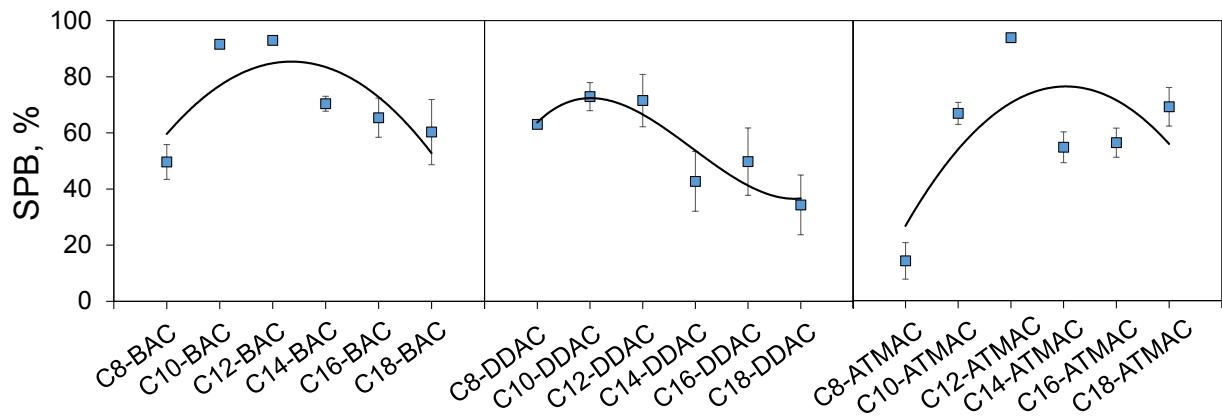


Figure S6. The relationship between binding affinities to serum proteins (SPB, %) and the length of the carbon chain. The error bars indicate the standard deviation of the triplicate tests. When not visible, error bars are contained within the data point.

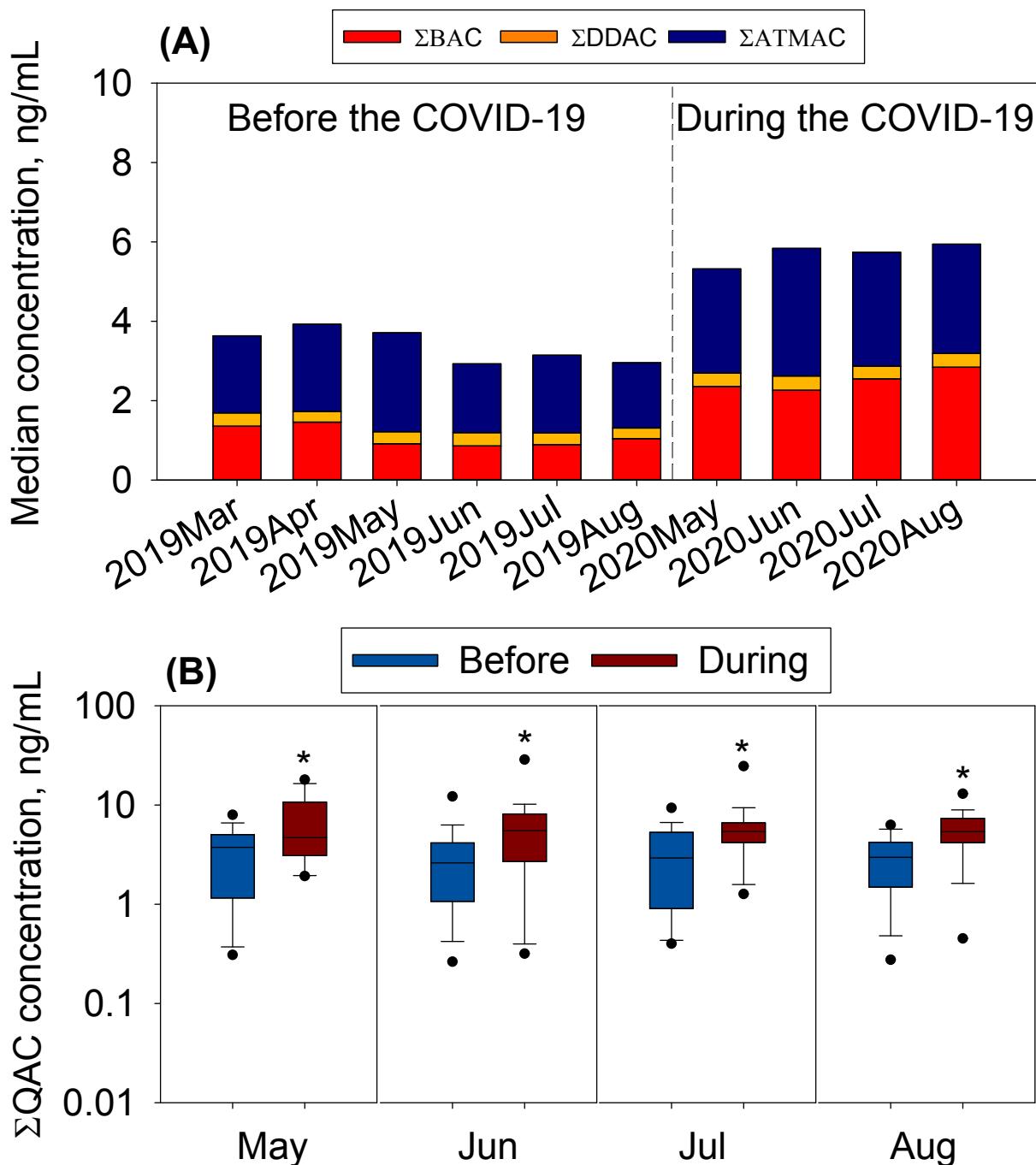


Figure S7. Median concentrations of Σ BACs, Σ DDACs and Σ ATMACs in serum (ng/mL) collected from before and during the COVID-19 pandemic (A). Total concentrations of the Σ QAC concentrations in serum collected in the same months in 2019 (before the pandemic) and in 2020 (during the pandemic) (B). Concentrations in B are shown as boxplots, representing the 25th and 75th percentiles; black lines represent the median; and the whiskers represent the 10th and 90th percentiles; and the dots indicate the 5th and 95th percentiles. The asterisks represent the statistical difference at $p < 0.05$ based on a Mann-Whitney test.